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Previews

Immunodominant-Peptide Recognition: Beta Testing TCRαβ

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In this issue, Ishizuka et al. (2008) define human T cell-receptor recognition of an immunodominant influenza A matrix peptide bound to human leukocyte antigen HLA-A*0201 in atomic detail, raising provocative questions about β chain function.

A key feature of αβ T cell receptor (TCR) recognition that emerged from the initial wave of structures of TCRs in complex with peptide-bound major histocompatibility complex (pMHC) is that each TCR assumes a common diagonal docking mode on the pMHC with its Vα domain docking over the N-terminal portion of the antigenic peptide and the Vβ domain docking over the C-terminal part (Rudolph et al., 2006; Teng et al., 1998). The inherent twist of the β sheet platform that forms the floor of MHC molecule's antigen-binding groove forces the two long α helices on the wall of the groove to each break into two segments, creating two peaks on the pMHC molecular surface. In order for the TCR to probe sufficiently deep into the MHC groove to make requisite contact with an antigenic peptide, a diagonal binding geometry is required to slot between the two peaks. Within this framework, binding variations in twist, tilt, and shift are observed. However, the general principles governing TCR-pMHC recognition and subsequent signal transduction remain essentially uncharted.

Ishizuka et al. (2008), in this issue of *Immunity*, now provides a close-up view of one important issue in the TCR field: the basis for immunodominance. Immunodominance refers to the phenomenon whereby after infection (or upon vaccination) the T cell immune response rapidly focuses on a very small number of epitopes out of an immensely large pool of potential epitopes. The mechanisms underlying T cell immunodominance are complex and poorly understood. Effective antigen processing, presentation copy number, and peptide-MHC binding kinetics, as well as T cell recognition, all affect the dominance of a very few peptides. Unique structural features of peptides presented on MHC surfaces can be important in selection of the corresponding immunodominant cytotoxic T lymphocyte (CTL) repertoire (Doherty et al., 2006; Meijers et al., 2005).

Ishizuka et al. (2008) investigate the human TCR-JM22 interaction with an immunodominant influenza matrix peptide (amino acids 58–66 with sequence GILGFVFTL) presented by the class I MHC molecule HLA-A*0201. This TCR incorporates the Vβ17 gene segment common to virtually all matrix-HLA-A*0201-specific T cells in humans (Ishizuka et al., 2008). The intriguing question asked is what makes a flat and featureless "vanilla" flu peptide immunodominant. In 2003, the same group determined the high-resolution crystal structure of Vβ17 TCR-HLA-A2-flu, revealing clues to the structural basis for predominant usage of the Vβ17 domain in HLA-A2-flu recognition. The highly selected amino acid sequence motif RSSY was identified in the CDR3β loop of the JM22 TCR as playing an essential role in peptide recognition. Particularly, the conserved Arg98β side chain on the loop docks into a notch formed between the peptide and HLA-A2. Moreover, side chains from CDR1β and CDR2β loops also hydrogen bond to the peptide, providing recognition specificity. The high-resolution JM22 TCR-pMHC-complex structure allowed them to scrutinize the interaction interface with 12 key contacting residues on JM22. In their new study, extensive kinetic and thermodynamic measurements were made with the employment of a series of rationally designed mutant JM22 TCRs. They systematically examine which residues are affinity determinants. At the same time, they determined more crystal structures of the JM22 TCR, wild-type or mutant, liganded and unliganded.

These biochemical, structural, and mutational analyses on the interaction interface identified a binding "hot spot" within the Vβ domain. Strikingly the majority of hot-spot residues are the germline-encoded Gln52β and Ile53β of CDR2β and Asp32β of CDR1β. Arg98β of CDR3β is the only crucial contributor from the nongermline-encoded hypervariable region. It is particularly interesting to note that the germline-encoded Gln52β and Asp32β residues are unique to the Vβ17 segment and that the Gln52Ala mutation completely abrogates any detectable binding. In the structure, the amido group of this Gln52β residue makes two hydrogen bonds to the peptide backbone, one to the carbonyl oxygen of Gly4 and the other to the amide group of Val6 of the peptide (Figure 1). The peptide actually arches out of the pMHC surface at the Gly4. The dihedral angles at the sharply bent position 4 are −92° and 5°, a conformation most favored for glycine to assume. It is conceivable that Gln52β has been preselected to recognize the backbone conformation of this particular antigenic peptide. In terms of sidechain recognition, the peptide is indeed featureless. N-terminal to Gly4, the peptide is entirely buried within the MHC molecule.

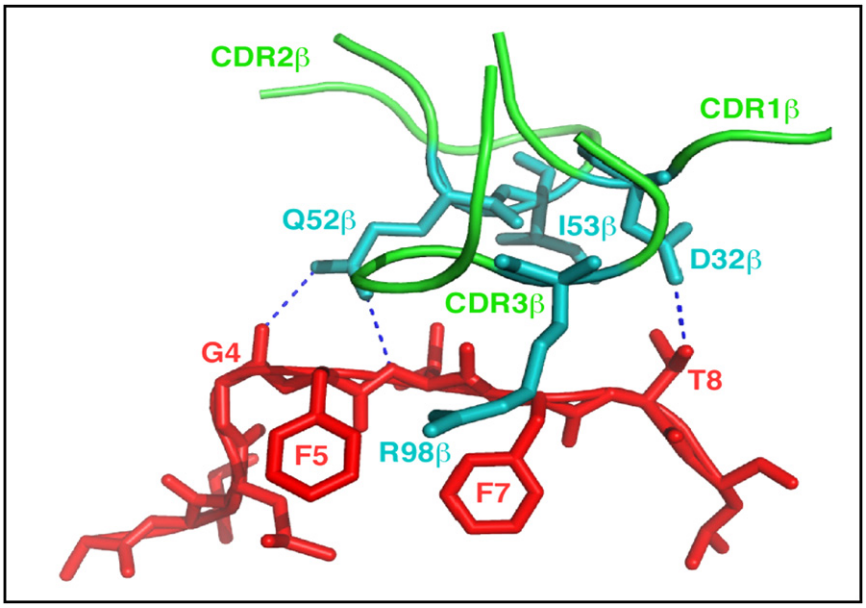

Figure 1. Immunodominance and β Chain Recognition of pMHC
Details of the JM22-HLA-A*0201-bound matrix-peptide interaction. The TCR Vβ CDR loops appear at the top in green and blue, whereas the influenza A peptide is shown at the bottom in red. Residues discussed in the text are labeled, and hydrogen bonds are shown as dotted lines.

The only prominent sidechain exposed from the pMHC surface to be seen by the TCR is that of Thr8. Its hydroxyl oxygen hydrogen bonds to Asp32β, whereas its methyl group makes a van der Waals contact to Ile53β. The peptide has two barely exposed phenol rings from Phe5 and Phe7. They contribute to notch-like binding by Arg98β as mentioned above. Furthermore, Arg98β forms three hydrogen bonds to the α2 helix of HLA and one to the peptide via a water molecule. Therefore, the biochemical data agree extremely well with the high-resolution structure, explaining the critical role played by the four residues in the hotspot of the conserved Vβ17 in recognition of the featureless immunodominant peptide.

Conservation among TCRs from T cells responding to an immunodominant pMHC ligand has been observed previously. Most extreme is the example of the human immune response in HLA-B8 individuals to Epstein-Barr virus latent antigen EBNA3A. CTLs with this specificity from unrelated individuals utilize the same TCR α and β pair. The structural basis for this selective dominance of a single αβ public clonotype has been elegantly described (Kjer-Nielsen et al., 2003). A third example, with conservation of the Vβ domain and a highly variable Vα usage, is found in B6 mice to the immunodominant D^b-restricted NP366-374 influenza A peptide from the nucleoprotein after PR8 viral infection. The repertoire is highly biased, with prominent TRBV13-1:TRBD1:TRBJ2-2 gene segment combination usage and selection of nine amino acid long CDR3β segments with a GxN sequence motif (Zhong et al., 2007, and references therein). It was suggested that conserved amino acid residues in the germline-encoded regions of a TCR Vβ subfamily member might favor key contacts with residues of a given pMHC complex, resulting in biased Vβ segment usage. Incorporation of such immunoprotective features of immune recognition into the germline then allows for ready selection of useful TCRs that are further improved upon by structurally relevant CDR3 segments. Certainly the example described by Ishizuka et al. (2008) is consistent with this notion. Of note, however, is that unlike with the highly biased TCR Vβ repertoire, the associated Vα repertoire specific for the NP366–374/D^b ligand remains extremely diverse, even after secondary viral infection.

In each of the examples above, the Vβ domain plays a key role in TCR-based recognition. The recent suggestion of potential germline-derived TCR-MHC pairwise interaction motifs, or "codons," linked to MHC restriction also pointed to highly biased Vβ domain selection (Feng et al., 2007). The observations raise the interesting possibility as to whether Vβ recognition itself can mediate a physiologic binding event within the immune system. If this were the case, then one could imagine how pairing of different Vα domains to the single Vβ domain could result in a broader range of T cell repertoire recognition against variant epitopes, thereby curtailing or at least minimizing escape from CTL control. This variable α pairing with a conserved β chain would not just broaden protection against the N-terminal portion of the MHC-bound peptide where the Vα domain largely interacts but would also impact the C-terminal end of the peptide, because the VαVβ pairing influences the configuration of Vβ CDR loops (Zhong et al., 2007). The concept that a single chain of an immune receptor exerts dominance has precedence in antibodies where the heavy chain is the major determinant of antigen affinity and specificity. Camelids, in fact, possess a functional class of antibodies devoid of light chain (Desmyter et al., 1996). Important questions are the following: Could the pre-TCR, consisting of a pTα-β heterodimer with an unpaired Vβ domain, functionally interact with MHC molecules in the thymus during β selection (von Boehmer, 2005) to begin the process of repertoire formation? Might β dominance in certain viral responses as shown here or in the alloreactive T cell response result from such a ligand-binding function? And, last but not least, are the subtle TCR conformational changes noted by Ishizuka et al. (2008) upon pMHC ligand binding a basis for selection or signaling? Time will tell. As always, quality studies raise more questions than they answer.

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Fas Bim Boom!

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New findings by Hughes et al. (2008), Hutcheson et al. (2008), and Weant et al. (2008) highlight the roles of apoptosis regulators Bim and Fas in the contraction phase of T cell responses and reveal consequences of failure of this process.

If the immune system were a television game show, the announcer might summarize the rules at the outset in this abbreviated way: "You know how we play: Each lymphocyte contestant recognizes a different antigenic determinant, and if they find it, they proliferate to make the most effective immune response to win the game. And that's all there is to it!" But real life is not reality TV, and a fundamental, if simple, problem with this "contest" quickly reveals itself—the expansion of lymphocytes in a few immune responses will overrun the system, precluding subsequent responses to new antigens, unless they can die rapidly enough to clear the way. And to make matters worse, any elicited responses that have an autoimmune component will wreak havoc. Now, three papers in this issue of *Immunity* (Hughes et al., 2008; Hutcheson et al., 2008; Weant et al., 2008) give us some idea of how catastrophic a failure to clear routine immune responses can be.

The rise and fall of an immune response is exemplified by the well-documented phenomenon of T cell clonal contraction, also called peripheral deletion. Numbers of responding T cells increase dramatically, peak, and then decline quickly (often at a point presumed to reflect removal of antigen), leaving a relatively small number of memory T cells. With an early model for such clonal contraction, it was found that defects in the death receptor, CD95 (Fas), and its ligand CD95-L (FasL), can play roles in this process (reviewed in Green et al., 2003), a finding that initially seemed to agree with several other observations: (1) Mice or humans with defects in either Fas ligand or its receptor have a marked lymphoaccumulation and often have accelerated autoimmune disease (Rieux-Laucat et al., 2003), (2) activation-induced cell death in T cells, as a consequence of restimulation of a previously activated T cell, can be due to Fas-FasL interactions (reviewed in Green et al., 2003), and (3) although apoptosis by this mechanism was the only form understood at the time, introduction of a *Bcl2* transgene (known to block apoptosis in some settings) expressed in T cells made the lymphoaccumulation much worse (Reap et al., 1995).

It emerged, however, that there were a number of problems with this view. Bcl-2 (and other antiapoptotic members of this family) inhibits the mitochondrial pathway of apoptosis, which is only sometimes important in death receptor (such as Fas)-mediated cell death and appears to have little or no role in Fas-mediated T cell death (Strasser et al., 1995). Further, the expansion of T cells in mice or humans lacking functional Fas or FasL is predominantly that of an unusual subset ($B220^+$, $CD3^+$, $CD4^-$, $CD8^-$) that does not arise in normal immune responses. But perhaps most importantly, the process of peripheral deletion was found to be far more dependent on the proapoptotic Bcl-2 family protein Bim, than on the death receptor, Fas (Hildeman et al., 2002). Bim is a trigger of the mitochondrial pathway of apoptosis and plays a prominent role in cell death caused by cytokine deprivation of T cells. Therefore, clonal contraction of T cells during an immune response appeared to be apoptosis due to limited growth and survival factors, rather than an active ligation of death receptors.

Three papers in this issue of *Immunity* (Hughes et al., 2008; Hutcheson et al., 2008; Weant et al., 2008) now help to resolve this. Mice deficient in both Fas function and Bim have normal development (including lymphoid development) but over time display enormous expansion of lymphoid (and some myeloid) cells. This includes profound increases in memory T cell pools (both central and effector), as well as B cells and macrophages. The $B220^+$, $CD3^+$ T cell subset seen in Fas-defective individuals was also greatly expanded. Stunningly, lymphoid organs in these mice can expand to represent half of the mass of the animal.

Clearly, this is an example of redundancy in a cell-death process, but who is backing up whom? Bim is a critical player in the apoptotic response to deprivation from survival factors, especially in hematopoietic cells, and accumulates under these conditions to engage the